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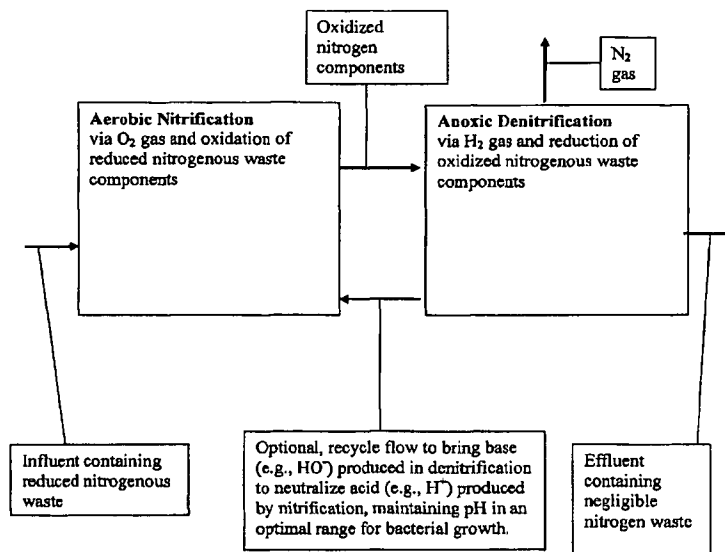
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(54) Title: METHODS AND SYSTEMS FOR TOTAL NITROGEN REMOVAL



(57) Abstract: A method and related apparatus for oxidation and reduction of a reduced aqueous nitrogen contaminant.



*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

## METHODS AND SYSTEMS FOR TOTAL NITROGEN REMOVAL

This application claims priority benefit of U.S. application serial no. 60/571,344, filed May 14, 2004, the entirety of which is incorporated herein by reference.

### Background of the Invention.

Excess nitrogen input to water bodies is becoming a substantial environmental concern. In general, elevated concentrations of nitrogenous species (N), such as ammonium or nitrate, spur eutrophication, which can lead to hypoxia, odors, color, and other undesirable water-quality changes. Ammonium also can be directly toxic to fish and exhibits a large oxygen demand. Furthermore, elevated levels of nitrate in drinking water cause methemoglobinemia in infants (Maxcy, 1950; Masters, 1998). As a result, the USEPA set the maximum containment level (MCL) for nitrate to 10 mg  $\text{NO}_3^-$ -N/l for drinking water (Masters, 1998). Ironically, the problems of N in water environments demand concentrations far lower than the MCL. In the future, wastewater discharge standards for total N may be as low as 1 to 3 mgN/L when the watershed is sensitive to eutrophication or hypoxia.

Ammonium is the most usual form of nitrogen pollution in wastewaters of various types. It is produced during the production of fertilizers and chemicals. As a result, large amounts of ammonium are present in agricultural runoff and discharges from the fertilizer industry. Ammonium pollution also is attributed to over-fertilization and intensive livestock farming. Another major source of ammonium nitrogen is domestic wastewater, since the N present in organic material in human wastes is ammonium or organic N that can be hydrolyzed to ammonium.

### Brief Description of the Drawings.

Figure 1A: A schematic diagram illustrating one or more of the present methods, in accordance with one or more embodiments of this invention.

Figure 1B: Schematic diagram of a hollow-fiber with the gas ( $\text{H}_2$  or  $\text{O}_2$ ) diffusing through the membrane wall and utilized by a biofilm on the outside, in accordance with one or more embodiments of this invention.

Figures 2A-B: Configurations of aerobic and anoxic MBfR component systems, in accordance with one or more embodiments of this invention.

Figure 3: Concentrations of N species during the initial 100 hours of treatment.

Figure 4: O<sub>2</sub> pressure effects on N species concentrations when the H<sub>2</sub> pressure was 2.5 psi. The first 100 hours represent a baseline operation.

Figure 5: H<sub>2</sub> pressure effects on N-species concentrations when the O<sub>2</sub> pressure was held constant at 3.6 psi.

Figure 6A: The effects of O<sub>2</sub> pressures on nitrate (NO<sub>3</sub><sup>-</sup>) concentration.

Figure 6B: The effects of O<sub>2</sub> pressures on nitrate (NO<sub>2</sub><sup>-</sup>) concentration.

Figure 7A: The effects of O<sub>2</sub> gas on NH<sub>4</sub><sup>+</sup> concentrations when O<sub>2</sub> <2.0 psi. (Note: The trend line in Figure 7A is exponential.)

Figure 7B: The effects of O<sub>2</sub> gas on NH<sub>4</sub><sup>+</sup> concentrations when O<sub>2</sub> >2.0 psi. (Note: The trend line in Figure 7B is linear.)

Figure 8: Percent total-N removal for constant pressure (2.5 psi) and an influent NH<sub>4</sub><sup>+</sup> concentration of 50 mgN/L.

Figure 9: Percent total-N removal at constant O<sub>2</sub> pressure (~3.6) and an influent NH<sub>4</sub><sup>+</sup> concentration of 50 mgN/L.

#### Summary of the Invention.

In light of foregoing, it is an object of the present invention to provide one or more methods, apparatus and/or systems for waste stream removal of nitrogenous contaminants, including without limitation ammonia and ammonium compounds, thereby addressing various concerns and issues relating to the prior art, including those outlined above. It will be understood by those skilled in the art that one or more aspects of this invention can meet certain objectives, while one or more other aspects can meet certain other objectives. Each objective may not apply equally, in all its respects, to every aspect of this invention. As such, the following objects can be viewed in the alternative with respect to any one aspect of this invention.

It is an object of the present invention to provide one or more methods for removal of reduced nitrogenous contaminants, including without limitation

ammonium compounds and related organic amine and quaternary amine contaminants, to levels meeting state and/or federal regulatory requirements.

It is another object of the present invention to provide for oxidation of reduced nitrogenous contaminants, alone or in conjunction with one or more influent oxidized nitrogen contaminants, in route to total nitrogen removal from an aqueous system.

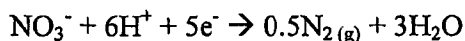
It is another object of the present invention to provide one or more apparatus and/or system configurations, for use in conjunction with the present methodologies, to effect simultaneous or coordinated oxidation and reduction of nitrogenous contaminants for N removal from an aqueous waste stream.

Other objects, features, benefits and advantages of the present invention will be apparent from the summary and its descriptions of various embodiments, and as apparent to those skilled in the art having knowledge of various oxidation/reduction reactions and waste treatment systems. Such objects, features, benefits and advantages will be apparent from the above as taken into conjunction with the accompanying examples, data, figures, and all reasonable inferences to be drawn therefrom.

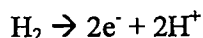
The present invention comprises one or more methods, together with apparatus and systems used in conjunction therewith, for nitrification and denitrification of nitrogenous waste components *en route* to total nitrogen removal from an aqueous waste stream. In accordance therewith, nitrification can be considered in the context of and illustrated by oxidation of an ammonium species (e.g.,  $\text{NH}_4^+$ ) to nitrate (e.g.,  $\text{NO}_3^-$ ) by nitrifying bacteria, as can be represented by the following non-limiting reaction:



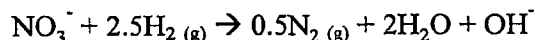
The oxidation of ammonium to nitrate can generate two acid equivalents ( $\text{H}^+$ ) per mole of nitrogen oxidized, with available molecular oxygen ( $\text{O}_2$ ), as shown. (2 mol  $\text{O}_2$ /mol  $\text{NH}_4^+$ ) Denitrification, conversely, can be considered as a reduction in which nitrate is reduced to nitrogen gas ( $\text{N}_2$ ), according to the non-limiting half reaction:



N<sub>2</sub> gas evolves, thereby eliminating N from the water. H<sub>2</sub> is microbiologically oxidized to provide the electrons (e<sup>-</sup>) to drive nitrate reduction, the oxidation half reaction for which is:



Combining the two half reactions, denitrification can be represented as



Overall, denitrification provides for reduction of nitrate to nitrogen gas, with one base equivalent (OH<sup>-</sup>) per mole of nitrogen reduced.

Accordingly, the present invention provides a method for removal of nitrogen components from an aqueous waste system. Such a method comprises (1) providing a system comprising at least one nitrification bacterium, and at least one denitrification bacterium; (2) introducing, to the system, an aqueous waste stream or water supply comprising a reduced nitrogenous component; and (3) contacting the system independently with oxygen and hydrogen. Aerobic conditions promote accumulation of nitrifying bacteria known in the art, while anoxic conditions, conversely, promote accumulation of denitrifying bacteria, also known in the art. Alternatively, such a system can be inoculated with nitrifying and/or denitrifying bacteria. Upon introduction of a reduced nitrogenous waste component, such as but not limited to an ammonium compound, oxidation and reduction proceed to effect removal of both reduced and oxidized nitrogen species from the aqueous system.

Alternatively, this invention can also provide a method of using denitrification bacteria to remove a reduced nitrogenous contaminant from a waste stream or water supply. Such a method comprises (1) providing a system comprising a first component comprising at least one nitrification bacterium and an aqueous volume comprising a reduced nitrogenous contaminant, and a second component comprising at least one denitrification bacterium; (2) contacting the first component with gaseous oxygen to oxidize the nitrogenous contaminant; (3) introducing and/or transferring the oxidized nitrogenous contaminant to the second component; and (4) contacting the second component with gaseous hydrogen. Certain embodiments of such a

method can be considered in the context of a reduced nitrogenous contaminant, such as but not limited to ammonia or an ammonium compound. Aerobic conditions can promote accumulation of a biomass comprising nitrification bacteria and oxidation to one or more oxidized (e.g., nitrate, nitrite) nitrogenous contaminants. Subsequent introduction of such an oxidized contaminant to the second component and a biomass comprising denitrification bacteria, as can be promoted under anoxic conditions, reduces the contaminant for waste stream removal.

Aerobic and anoxic conditions can be changed and/or optimized, depending upon a particular waste stream and nitrogen species/content, by variation of oxygen and hydrogen pressures, respectively. (See, Examples 2-4, below.) Such coordinated oxidation and reduction can reduce effluent concentrations to less than about 1mgN/L for a particular reduced or oxidized nitrogen contaminant. Likewise, total nitrogen effluent can be reduced to concentrations less than about 2mg/L, in accordance with applicable state and federal regulatory requirements. Simultaneous, sequential or coordinated oxidation and reduction also provide for neutralization of an acid by-product of nitrification by a base by-product of denitrification, thereby minimizing or eliminating the need for extraneous system pH control. In addition to the benefits of acid/base neutralization, waste water recirculation can afford various other advantages. For example, repeat processing or cyclization can enhance contaminant removal. Alternatively, recirculation can be used to control water flow velocity past one or both of the nitrification and denitrification reactor (i.e., NR and DR) components, independently from the influent flow rate. A high flow velocity in either component can promote a strong, dense biofilm and mass transfer of reduced or oxidized nitrogen contaminant to the biofilm. Such aspects and related benefits of the present methodologies can be considered with reference to Figures 1A-B and 2A-B, below, such figures non-limiting with respect to any particular method/process parameter or system component material, dimension or configuration provided therein.

Such methods can be employed without restriction to any one apparatus or component configuration. Nonetheless, one or more methods of this invention can be used in conjunction with an apparatus comprising (1) a first component comprising at least one hollow membrane, each of which is in fluid communication with an oxygen source; and (2) a second component in fluid communication with the first component, the second component comprising at least one hollow membrane, each of which is in fluid communication with a hydrogen source. In certain embodiments, the membrane can comprise inner and outer layers having a first density and a layer therebetween having a second density greater than the first density. In various preferred embodiments, while the former layers can be porous, the latter layer can be substantially non-porous to reduce or avoid sparging of either hydrogen or oxygen, or conditions otherwise deleterious to biomass accumulation.

Likewise, without restriction to any one waste treatment system, apparatus or component configuration, this invention can also provide a method for oxidation of a reduced nitrogenous contaminant. Such a method can comprise: (1) providing a system comprising a component comprising a membrane and nitrification bacteria thereon, and an aqueous volume comprising a reduced nitrogenous contaminant; and (2) contacting the system with oxygen to oxidize the nitrogenous contaminant. As discussed elsewhere herein, such a system can be inoculated with bacteria, or aerobic conditions can selectively promote accumulation of a biomass comprising nitrification bacteria. In certain embodiments, such a system can comprise at least one membrane component in fluid communication with an oxygen source. Such a component can be arranged and configured as described above, or in one or more of the incorporated references cited below. A corresponding oxidized contaminant can then, without limitation, be introduced to a second system or component comprising denitrification bacteria, as described elsewhere herein, to reduce the contaminant for subsequent removal. Alternatively, such an oxidation method and/or use of nitrification bacteria can be used in conjunction with any reduction or denitrification process known in the art.



Detailed Description of Certain Embodiments.

System/apparatus component configurations, as can be employed, are described more fully in United States Pat. No. 6,387,262 and pending application serial no. 10/930,051 filed August 30, 2004, each of which is incorporated herein by reference in its entirety. Apparatus components and configurations, as enumerated and provided in the figures and corresponding specification of such incorporated references, can be used effectively within the context of this invention. Accordingly, in certain embodiments, a membrane biofilm reactor (MBfR) can deliver  $H_2$  or  $O_2$  gas to denitrifying or nitrifying bacteria, respectively, by diffusing through the wall of a bubble-less membrane. A bacterial biofilm naturally develops on the outside of the corresponding membrane wall. Nitrification bacteria utilize  $O_2$  to facilitate oxidation of a reduced nitrogen component to, e.g.,  $NO_3^-$ . With regard to denitrification, bacteria in the biofilm remove electrons from  $H_2$  diffused to the outside of the membrane to reduce  $NO_3^-$  to  $N_2$  gas.

Nitrifying bacteria are known in the art, representative species of which include but are not limited to *Nitrosomonas europaea*, *Nitrosomonas eutropha*, *Nitrospira briensa*, *Nitrobacter agilis*, and *Nitrospira marina*. Likewise, denitrification bacteria are also available and well-recognized. Although not limited to autotrophic (i.e., hydrogen-oxidizing) species, representative examples of such organisms include but are not limited to *Paracoccus denitrificans*, *Alcaligenes eutrophus*, *Pseudomonas pseudoflava*, and *Ralstonia eutropha*. Such bacteria can be introduced to respective oxidation and reduction reactor components using a corresponding inoculant from an existing nitrogen treatment system or through selective reactor biomass accumulation under the aerobic and anoxic conditions induced therein. (See, e.g., the aforementioned incorporated '262 patent and the co-pending '051 application.)

An  $H_2$ -based MBfR is useful for reducing  $NO_3^-$  (or, e.g., nitrite,  $NO_2^-$ ) to  $N_2$  gas.  $H_2$  is the least expensive electron donor that can be purchased in bulk quantity, and it is non-toxic to humans. Using  $H_2$  eliminates the need to supply an organic-C source, which is expensive, often introduces handling and safety

problems, and can easily be under-dosed or over-dosed. Delivering  $H_2$  by diffusion through the membrane wall eliminates the risk of over-dosing, and under-dosing is simply and rapidly remedied by increasing the  $H_2$  pressure to the membrane. Having biofilm on the membrane wall ensures nearly 100% efficiency of  $H_2$  use improving the cost-effectiveness of the process. A benefit of using a bubble-less membrane is safe delivery of  $H_2$  gas. With other delivery methods, hydrogen gas can accumulate in the gas phase to create an explosive environment and thus a substantial safety risk (the explosive range for hydrogen is about 4 to 74.5% in air). Bubble-less delivery and immediate  $H_2$  consumption by the biofilm preclude formation of an explosive gas phase.

For total nitrogen removal, any reduced nitrogen component (e.g., ammonia or an ammonium species) present is oxidized to  $NO_3^-$  (or nitrite,  $NO_2^-$ ) by aerobic nitrifying bacteria. An MBfR system and apparatus of this invention can also be used for both denitrification and nitrification. As illustrated in Figure 1B,  $O_2$  gas can be delivered by diffusion through a membrane wall. To prevent bubbling, a fiber component can comprise a 1- $\mu m$  thick nonporous, hydrophobic polyurethane layer located between microporous polyethylene walls. Fibers of the sort useful in systems/apparatus components of the sort illustrated in Figure 1B are manufactured by Mitsubishi Rayon (Model MHF 200TL) and are available as a composite. A dense, non-porous layer allows the creation of a high driving force for gas dissolution without premature bubble formation, thereby promoting efficient oxygen use and accumulation of desired nitrifying bacteria. The hollow fibers can be sealed on one end and open to pressurized oxygen (or hydrogen) on the opposite end. Other fiber configurations known in the art can provide bubble-less operation. Induced biofilm accumulation facilitates electron transport between an aqueous nitrogen component (e.g.,  $NH_4^+$  or  $NO_3^-$ ) and a gas introduced (e.g.,  $O_2$  or  $H_2$ ).

Accordingly, an aerobic and anoxic system of this invention can comprise two MBfRs for integrated operation. One MBfR component is supplied with  $O_2$  gas to achieve nitrification (NR). The other MBfR

component is supplied with  $H_2$  gas to achieve denitrification (DR) -- to approach complete N removal. One such aerobic and anoxic component configuration is shown in Figure 2A. In certain embodiments, each column has 32 hollow fibers of length 25 cm, giving a total biofilm surface area of  $70.6 \text{ cm}^2$  for each MBfR component. The volume of each MBfR is approximately  $10 \text{ cm}^3$ . A useful influent pumping rate is set to  $0.11 \text{ cm}^3/\text{min}$ , giving a detention time for the MBfR system of approximately 3 hours. The recycle flow rates shown in Fig. 2A (e.g., a pump at  $50 \text{ ml}/\text{min}$  and a pump at  $150 \text{ ml}/\text{min}$ ) can be used to link the two MBfRs and to provide adequate mixing and ensure adequate oxidation/reduction. An alternate component configuration is shown in Fig. 2B and provides for optional, extraneous buffer capability. Introduction of a buffer component (i.e., for acid and/or base neutralization) may be desired for treatment of waste streams having elevated levels of nitrogenous waste. Likewise, depending on volume or contaminant levels, multiple hollow fiber membrane components can be employed with the nitrification and/or denitrification aspects of this invention.

Various other embodiments of this invention can be considered with reference to the apparatus components and materials of the systems provided in the aforementioned incorporated '262 patent and co-pending '051 application, in particular Figures 1-2 thereof. Alternatively, membranes useful in conjunction with this invention can comprise one or more sheets or another material arrangement known in the art. Regardless, whether fiber, sheet or another such arrangement, membrane components can be positioned randomly or in a predetermined spatial configuration, such as without limitation in a row configuration, or in a configuration of rows and columns. With respect to the latter, one or more grid support components can be used to position the fibers or sheets at points with respect to one or more oxygen or hydrogen sources. Positional configuration of such components can define intercomponent spaces for movement of water therethrough. Without limitation, such spacing allows movement of water-borne contaminants transverse to membrane components, as is especially useful in the treatment of waste water and other aqueous

systems comprising solid particulate matter. Such components and related apparatus configurations are described more fully in co-pending application serial no. 10/876,745, in particular Figures 2A-C thereof, such application incorporated herein by reference in its entirety.

Examples of the Invention.

The following non-limiting examples and data illustrate various aspects and features relating to the methods and/or apparatus of the present invention. In comparison with the prior art, the present methods and apparatus provide results and data that are surprising, unexpected and contrary thereto. While the utility of this invention is demonstrated through the use of several apparatus configurations and simulated nitrogenous waste streams in conjunction therewith, it will be understood by those skilled in the art that comparable results are obtainable with various other apparatus and waste stream compositions (e.g., organic amines and ammonium compounds), as are commensurate with the broad scope of the present methodology. As such, the present invention contemplates an apparatus/system with aerobic and anoxic components for sequential or simultaneous nitrification and denitrification over a range of oxygen and hydrogen gas delivery rates and pressures, to achieve effluent concentrations of less than about 1 mg/L for both reduced and oxidized nitrogen waste components, and substantially total nitrogen removal for influent waste streams containing ammonia.

A medium used in the following examples, as would be understood in the art to demonstrate the wide utility of this invention, was synthetic wastewater with the composition shown in Table 1. The ammonium concentration was 50 mg  $\text{NH}_4^+\text{-N/l}$ . The buffer capacity of the water was added in the form of 0.252 g/l  $\text{HCO}_3^-$ , 0.136 g/l  $\text{H}_2\text{PO}_4^-$ , and 1.134 g/l  $\text{HPO}_4^{2-}$ . The medium was contained in a  $\text{N}_2$ -gas-sparged bottle to prevent uncontrolled changes in the carbonate system.

**Table 1:** Composition of the synthetic-wastewater medium

Media Ingredient	Quantity
Distilled Water	5 L
NaHCO <sub>3</sub>	1.26 g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	1.179 g
KH <sub>2</sub> PO <sub>4</sub>	0.681 g
NaHPO <sub>4</sub>	5.678 g
Trace Minerals	7.5 ml
Ca-Fe Solution	7.5 ml
MgSO <sub>4</sub> ·7H <sub>2</sub> O	7.5 ml

The parameters measured were NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup>, and pH. The analytical methods are summarized in Table 2. Each method was calibrated against known standards to ensure measurement accuracy. A Spectronic Spec 20 was used to measure the absorbance of the NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, and NO<sub>2</sub><sup>-</sup> samples.

**Table 2:** Summary of analytical methods

Parameter	Method	Reference	Range	Std. Dev.
NO <sub>3</sub> <sup>-</sup>	Chromotropic Acid Method	Hach Method 10020	0-30.0 mg/l NO <sub>3</sub> <sup>-</sup> -N	+/- 1%
NO <sub>2</sub> <sup>-</sup>	Diazotization Chromotropic Acid Method	Hach Method 8507	0-0.500 mg/l NO <sub>2</sub> <sup>-</sup> -N	+/- 2.3%
NH <sub>4</sub> <sup>+</sup>	Salicylate Method	Hach Method 10031	0-50.0 mg/l NH <sub>3</sub> -N	+/- 2%
pH	Electrometric Method	Standard Methods (1998) – 4500-H <sup>+</sup>	0-14 pH	+/- 0.1 pH

The medium was fed continuously for several weeks while biofilms accumulated on the outside of the hollow fiber membranes (see, e.g., Fig. 1B and Fig. 2A) and to establish baseline performance(s). Once a visible layer of biofilms was present and N removal was stable for the baseline conditions, experiments were run to determine the nitrogen removal under different gas pressure conditions in the MBfRs. Under constant H<sub>2</sub> pressure (2.5 psi), the removal of nitrogen was determined for the following O<sub>2</sub> pressures: 1.25 psi, 2.0 psi, and 6.0 psi. Under constant O<sub>2</sub> pressure (3.6 psi), the removal of

nitrogen was determined for 1.25 psi and 5.0 psi H<sub>2</sub> pressure. The system was allowed to reach steady-state performance before changing O<sub>2</sub> or H<sub>2</sub> pressure. The H<sub>2</sub> gas pressure was originally maintained at 2.5 psi while changing the O<sub>2</sub> pressure. The H<sub>2</sub> pressure was then varied with the O<sub>2</sub> pressure maintained at 3.6 psi. At least 3 samples were taken for each gas pressure, with a minimum of 9 hours between samples. Each sample was filtered (0.45-μm membrane filter) to remove any suspended microbes detached from the biofilm.

#### Example 1

##### *Baseline N Removal*

The first 100 hours of results in Figure 3 show the baseline removal of N when the H<sub>2</sub> pressure was 2.5 psi and the average O<sub>2</sub> pressure was 2.5 psi. The concentrations of all N species were low: near to or less than 1 mgN/L for NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>, with NO<sub>2</sub><sup>-</sup> nearly zero. The total N concentration (i.e., the sum of NH<sub>4</sub><sup>+</sup> + NO<sub>3</sub><sup>-</sup> + NO<sub>2</sub><sup>-</sup> as N) always was less than 3 mgN/L and typically was less than 2 mgN/L. These results clearly demonstrate that the aerobic and anoxic MBfR system can achieve very low concentrations of all N species and total N.

#### Example 2

##### *Changing O<sub>2</sub> Pressure*

Figure 4 also illustrates the general trend for nitrate and ammonium as the oxygen pressure was changed over time (at 110 to 280 hours). The ammonium concentration was greatly increased by a low oxygen pressure (1.25 psi). This was due to oxygen limitation of the nitrification reaction in the aerobic MBfR. On the other hand, the nitrate concentration was high when the oxygen pressure was increased (6 psi), presumably because dissolved oxygen carry over from the aerobic MBfR to the anoxic MBfR inhibited denitrification of NO<sub>3</sub><sup>-</sup> to N<sub>2</sub>. The nitrite concentration was small and was largely unaffected by the gas pressures under the conditions utilized. The total nitrogen concentration mirrored the concentration of an N species that had an increased concentration due to the change in O<sub>2</sub> pressure.

The results in Figure 4 document the anticipated trends with O<sub>2</sub> availability. Too little O<sub>2</sub> availability slowed nitrification in the aerobic MBfR, leaving NH<sub>4</sub><sup>+</sup> unoxidized. Too much O<sub>2</sub> availability in the aerobic MBfR led to dissolved oxygen carry over, which inhibited NO<sub>3</sub><sup>-</sup> reduction in the anoxic MBfR. Thus, the integrated aerobic and anoxic system behaved in a predictable manner, and showed that controlling the O<sub>2</sub> pressure to the aerobic MBfR could be used to optimize total-N removal. Proper control of the O<sub>2</sub> pressure allowed excellent removal of all N species (< 1 mgN/L) and total N (< 3 mgN/L), as shown by the results in the first 100 hours.

### Example 3

#### *Changing H<sub>2</sub> Pressure*

Figure 5 presents the results for variation of H<sub>2</sub> pressure while keeping the O<sub>2</sub> pressure at 3.6 psi. Varying the hydrogen pressure did not alter the concentrations as dramatically as did changing the oxygen pressure. The ammonium and nitrite concentrations were almost completely unaffected by the H<sub>2</sub> pressure changes. On the other hand, the nitrate concentrations increased substantially when the H<sub>2</sub> pressure was low (1.25 psi). The total-N concentration paralleled the NO<sub>3</sub><sup>-</sup> concentration.

The results in Figure 5 document the anticipated trends with H<sub>2</sub> availability. Too little H<sub>2</sub> availability slowed denitrification in the anoxic MBfR, leaving NO<sub>3</sub><sup>-</sup> unreduced. However, increasing the H<sub>2</sub> availability in the anoxic MBfR appeared not to have a negative impact on nitrification in the aerobic MBfR. This good result may have occurred because H<sub>2</sub> has low water solubility, which precludes significant carry over to the aerobic MBfR. Again, the integrated aerobic and anoxic system behaved in a predictable manner, to show that controlling the H<sub>2</sub> pressure to the anoxic MBfR can be used to achieve total-N removal. Proper control of the H<sub>2</sub> pressure allows excellent removal of all N species and total N, as shown by the results in the first 100 hours (Fig. 3).

#### Example 4

##### *Summary of Gas-Pressure Effects*

Figures 6A-B summarize how the O<sub>2</sub> pressure affected the NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> concentrations for all experiments. Clearly, nitrate increased with increasing O<sub>2</sub> pressure (Fig. 6A) due to oxygen carry over from the aerobic MBfR. On the other hand, the NO<sub>2</sub><sup>-</sup> concentration was hardly affected by O<sub>2</sub> pressure (Fig. 6B). As shown in Figure 5, the H<sub>2</sub> pressure strongly controlled the effluent NO<sub>3</sub><sup>-</sup> concentration, and pressures greater than 1.25 psi H<sub>2</sub> significantly increased nitrate removal.

#### Example 5

Figures 7A-B shows that the ammonium concentration was drastically decreased when the O<sub>2</sub> pressure was greater than 1.25 psi. At the low O<sub>2</sub> pressures (Fig. 7A), oxygen was insufficient to allow full nitrification in the aerobic MBfR. As a result, the oxidation rates for NH<sub>4</sub><sup>+</sup> were low. Once the O<sub>2</sub> pressure was greater than 2.5 psi (Figure 7B), NH<sub>4</sub><sup>+</sup> removal did not correlate strongly with O<sub>2</sub> pressure, although there is a slight downward sloping trend between the two variables (R = -0.23). As shown in Fig. 5, NH<sub>4</sub><sup>+</sup> did not correlate to H<sub>2</sub> pressure.

#### Example 6

##### *Summary of Nitrogen Removal Efficiency*

Figures 8 and 9 summarize the total-N removal efficiencies when the influent had a fixed N concentration of 50 mgN/L, all as NH<sub>4</sub><sup>+</sup>. At constant H<sub>2</sub> pressure to the anoxic MBfR (2.5 psi), the total nitrogen removal (Fig. 8) was greatest for the intermediate O<sub>2</sub> pressure of 2.0 psi to the aerobic MBfR (97.1%). This corresponds to an effluent total N of only 1.5 mgN/L. At 6.0 psi, the nitrogen removal was 91.9%, and decrease was due to increased NO<sub>3</sub><sup>-</sup>. Modest removal was achieved with at 1.25 psi, with a nitrogen removal of 58.1% and mostly NH<sub>4</sub><sup>+</sup> in the effluent. Again, controlling O<sub>2</sub> availability in the aerobic MBfR can be used to achieve very high total-N removal.

At constant O<sub>2</sub> pressure (~3.6 psi), under the conditions/apparatus used the total nitrogen removal was optimized at 5 psi for H<sub>2</sub> (95.1%, giving an



effluent total N of 2.5 mgN/L). At 1.25 psi, the nitrogen removal was slightly less, 92.2%, due to less denitrification. Controlling  $H_2$  availability in the anoxic MBfR can be effective, but maximum total-N removal appears more directly related to  $O_2$  availability in the aerobic MBfR.

\* \* \*

The preceding examples show that an aerobic and anoxic MBfR system, in accordance with this invention, can provide nearly complete removal of total N. Reduced N contaminant(s) and a concentration of ~50 mgN/L, as used herein, are conditions typical for municipal wastewater and relevant to many other wastewaters. Under aerobic conditions ( $O_2$  gas) nitrifying biofilm oxidized  $NH_4^+$  to  $NO_3^-$  and  $NO_2^-$ , while under anoxic conditions ( $H_2$  gas) denitrifying biofilm reduced the  $NO_3^-$  and  $NO_2^-$  to  $N_2$  gas. Since the nitrifying and denitrifying bacteria are autotrophic, no carbonaceous energy source was required, thereby eliminating significant material costs and process hazards.

Total-N removal can be influenced by the  $O_2$  pressure in that insufficient  $O_2$  pressure decreased nitrification of  $NH_4^+$  to  $NO_3^-$  or  $NO_2^-$ , while high  $O_2$  pressure can inhibit denitrification of  $NO_3^-$  to  $N_2$  gas in the anoxic component. Total nitrogen removal is less strongly influenced by  $H_2$  pressure, although increasing  $H_2$  pressures can provide better denitrification without affecting nitrification.

While the principles of this invention have been described in connection with specific embodiments, it should be understood clearly that these descriptions are added only by way of example and are not intended to limit, in any way, the scope of this invention. For instance, simultaneous or coordinated nitrification and denitrification are demonstrated, here, in the context of total N removal, but this invention can also be used in conjunction with various other waste streams, whenever reduced and oxidized contaminants co-exist. Reduced contaminants can further include, without limitation, all organic matter, and organic nitrogen compounds, whereas oxidized contaminants can, likewise without limitation, include perchlorate, chlorate, chlorite, chromate, selenate, selenite, bromate, sulfate, sulfite, uranium, plutonium, neptunium and

chlorinated organic compounds such as but not limited to trichloroethene and trichloroethene.

We claim:

1. A method for removing a reduced nitrogenous component from an aqueous system, said method comprising:  
providing a system comprising nitrifying bacteria, denitrifying bacteria, and an aqueous reduced nitrogen component;  
contacting said system independently with oxygen and hydrogen; and  
oxidizing and reducing said component to generate nitrogen gas.
2. The method of claim 1 wherein aerobic conditions promote system accumulation of nitrifying bacteria, and anaerobic conditions promote system accumulation of denitrifying bacteria.
3. The method of claim 2 wherein oxygen pressure and hydrogen pressure are independently varied.
4. The method of claim 1 wherein said reduced nitrogen component is sequentially oxidized and reduced, said oxidation producing an acidic by-product, and said reduction producing a basic by-product, said acidic and basic by-products at least partially neutralizing one another.
5. The method of claim 1 wherein said reduced nitrogen component is selected from ammonia, an ammonium ion and a nitrogenous organic compound and combinations thereof.
6. The method of claim 5 wherein said reduced nitrogen component is oxidized to at least one of a nitrite and a nitrate.
7. The method of claim 6 wherein said reduced nitrogen component has an effluent concentration less than about 1mgN/L.
8. The method of claim 1 wherein said system comprises an influent oxidized nitrogen component.
9. The method of claim 8 wherein said reduced and oxidized nitrogen components have a total effluent concentration less than about 2mgN/L.
10. The method of claim 1 wherein anaerobic conditions promote system accumulation of hydrogen-oxidizing bacteria.

11. The method of claim 10 wherein said system comprises perchlorate, chlorate, chlorite, selenate, selenite, bromate, sulfate, sulfite, uranium, plutonium neptunium, and chlorinated organic compounds, and combinations of said compounds.
12. A method of using denitrifying bacteria to remove a reduced nitrogen contaminant from an aqueous system, said method comprising:
  - providing an aqueous system comprising a first component comprising at least one nitrification bacterium, a second component comprising at least one denitrification bacterium, and a reduced nitrogenous contaminant;
  - contacting said first component with gaseous oxygen;
  - introducing said oxidized nitrogenous contaminant to said second component; and
  - contacting said second component with gaseous hydrogen.
13. The method of claim 12 wherein aerobic conditions promote system accumulation of a nitrification bacterium, and anaerobic conditions promote system accumulation of a denitrification bacterium.
14. The method of claim 13 wherein said bacteria are autotrophic.
15. The method of claim 13 wherein said gaseous oxygen is provided substantially without sparging.
16. The method of claim 12 wherein said nitrogenous contaminant is sequentially oxidized and reduced, said oxidation producing an acidic by-product, and said reduction producing a basic by-product, said acidic and basic by-products at least partially neutralizing one another.
17. The method of claim 16 comprising repeated contact of said aqueous volume with said first and second components.
18. The method of claim 12 wherein said reduced nitrogenous contaminant is selected from ammonia, an ammonium ion, and an organic compound, and combinations thereof.
19. The method of claim 18 wherein said contaminant has an effluent concentration less than about 1mgN/L.
20. A membrane biofilm reactor apparatus, comprising:

a first component comprising at least one hollow membrane, each said fiber in fluid communication with an oxygen source;

a second component in fluid communication with said first component, said second component comprising at least one hollow membrane, each said membrane in fluid communication with a hydrogen source; and

nitrifying bacteria on said first component, and denitrifying bacteria on said second component.

21. The apparatus of claim 20 wherein each said membrane comprises inner and outer layers having the first density, and a layer therebetween having a second density greater than first said density.

22. The apparatus of claim 21 wherein said layer between said inner and outer layers is substantially non-porous.

23. The apparatus of claim 20 wherein each of said first and second components comprises a plurality of hollow fiber membranes.

24. A method for oxidation of a reduced nitrogenous contaminant, said method comprising:

providing a system comprising a component comprising a membrane and nitrification bacteria thereon, and an aqueous volume comprising a reduced nitrogenous contaminant; and

contacting said component with oxygen.

25. The method of claim 24 wherein said nitrogenous contaminant is selected from ammonia, an ammonium ion, and an organic compound, and combinations thereof.

26. The method of claim 24 wherein said nitrification bacteria are autotrophic.

27. The method of claim 24 wherein said component comprises at least one hollow fiber membrane in fluid communication with an oxygen source.

28. The method of claim 24 wherein said oxidized contaminant is reduced.

29. The method of claim 28 wherein said oxidized contaminant is contacted with a second system comprising a component comprising a membrane and denitrification bacteria thereon, said second system component in fluid communication with a hydrogen source.

30. The method of claim 29 wherein aerobic conditions promote system accumulation of nitrification bacteria, and anaerobic conditions promote system accumulation of denitrification bacteria.

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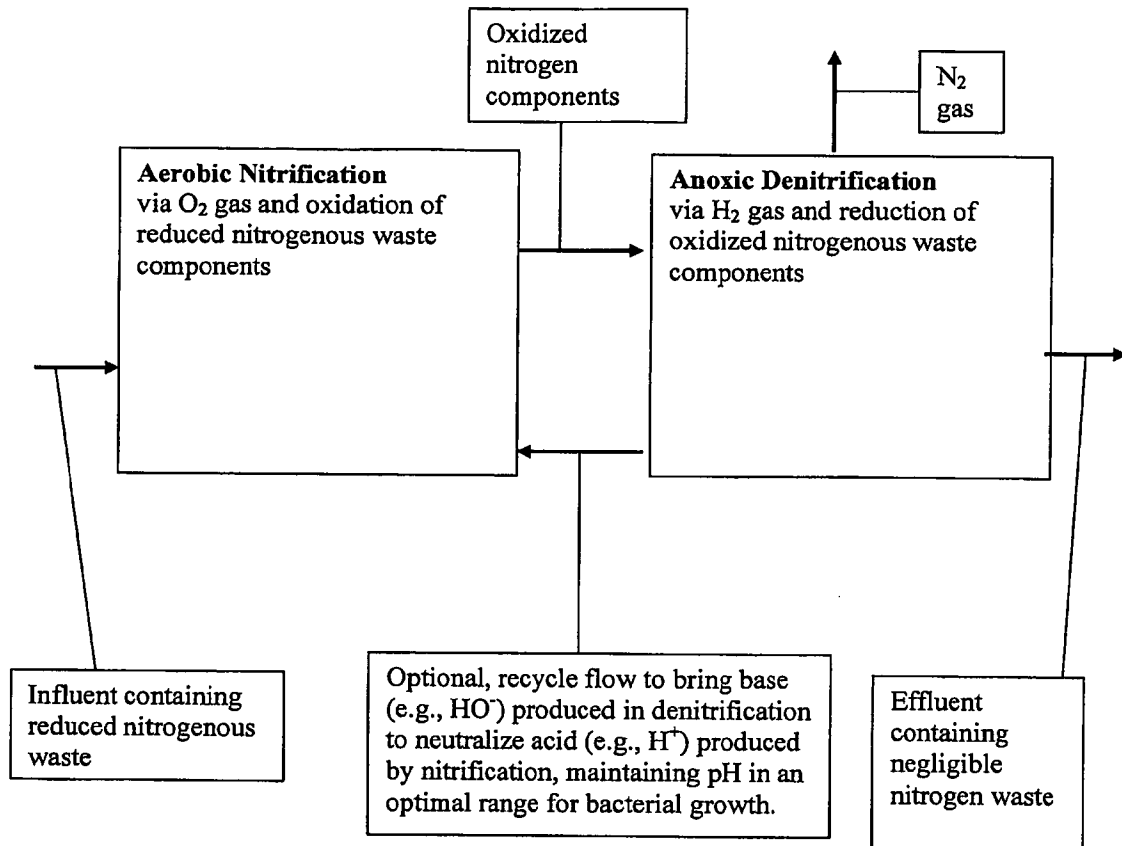
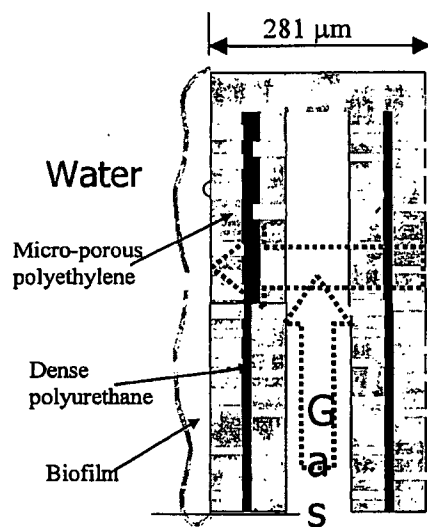


Figure 1A

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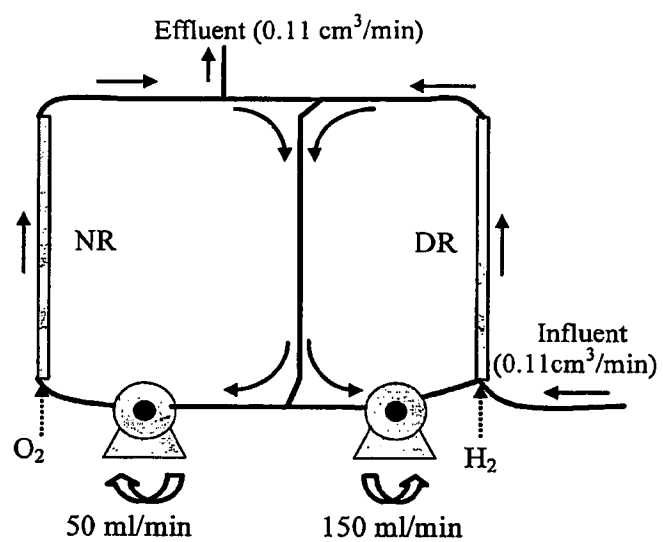
Figure 1B





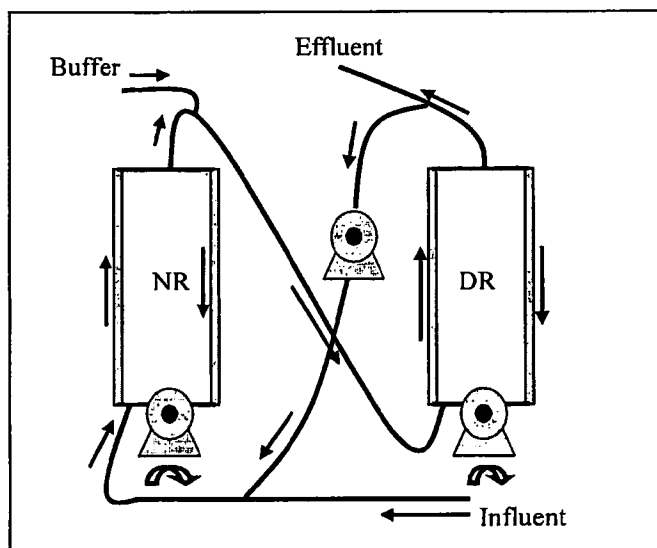
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Figure 2A



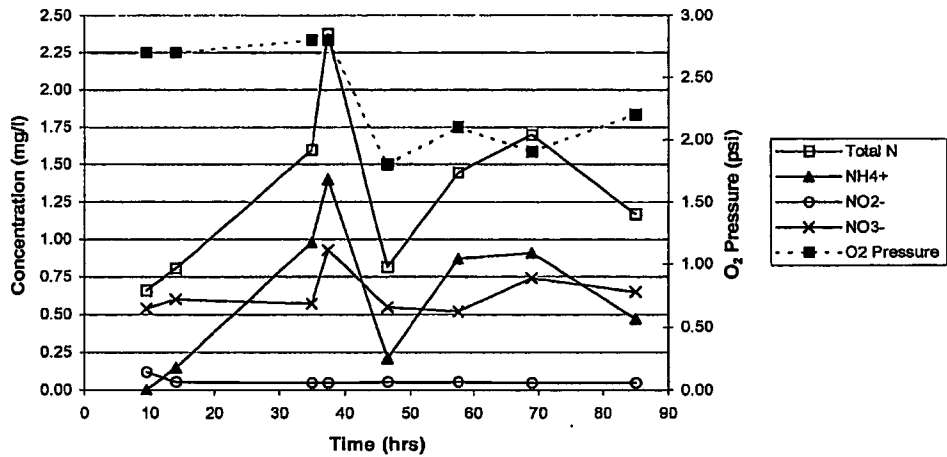
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Figure 2B



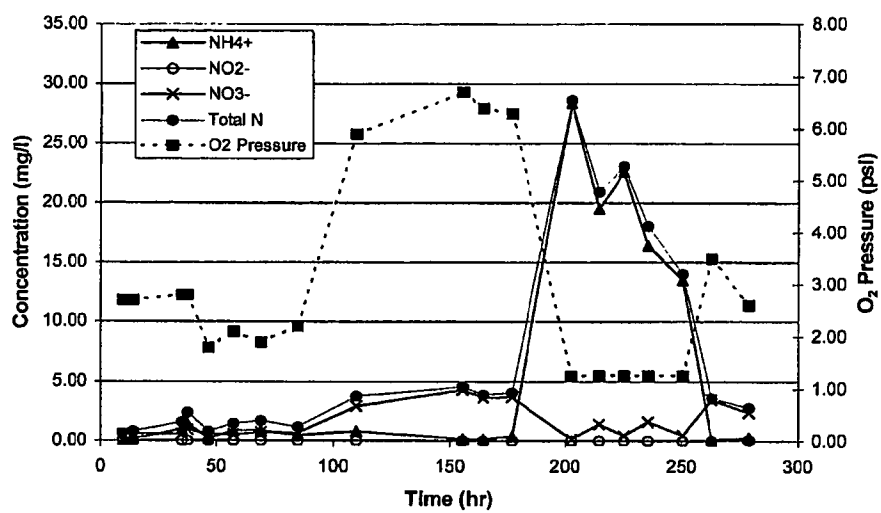
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Figure 3



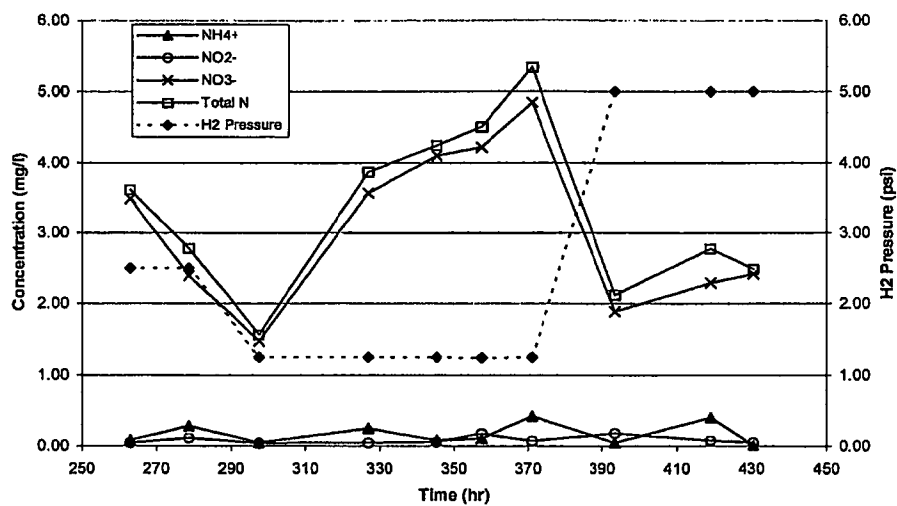
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Figure 4



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Figure 5



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Figure 6A

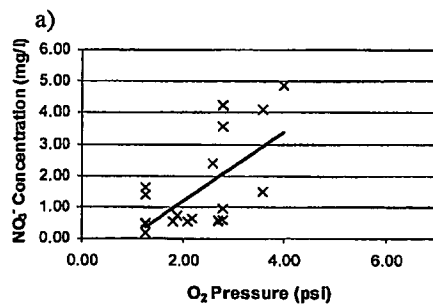
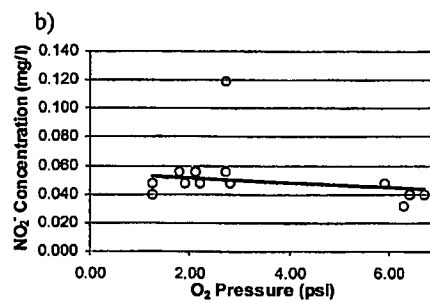


Figure 6B



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Figure 7A

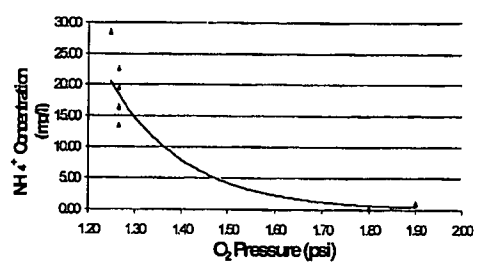


Figure 7B

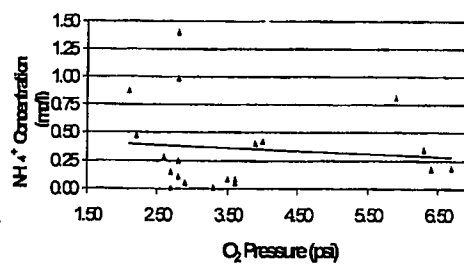


Figure 8

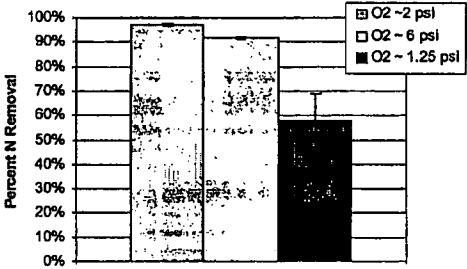


Figure 9

